impact (2-14 MPa) mechanical injury in a mechanical testing device. GAG alternation was determined by safrain O and DMB assay and Cell viability was analyzed by live dead assay. The type of cell death was characterlized by immunohistochemistry using active caspase-3 and finally pan-caspase inhibitor Z-VADfmk was suspended in culture media and determine the prevention of apoptosis after injury.

Results: GAG depletion did not lead to an increase in cell death directly when cartilage explants were observed for up to 2 weeks in culture. Mechanical injury in control explants did not cause an immediate change in cell viability, but viability decreased during the subsequent culture period to approximately 53.2% on day 3. In CABC-treated explants mechanical injury caused an immediate reduction in cell viability (from 84.6% to 71.0%). This immediate cell death was not inhibited by preincubation with the pan-caspase inhibitor Z-VADfmk, suggesting cell necrosis. During subsequent culture the viability in these explants decreased further to 50.5% on day 3. The second wave of cell death was almost completely inhibited by Z-VADfmk in CABC-treated explants. This second wave of cell death was also associated with activation of caspase-3, suggesting apoptotic mechanisms of cell death. When CABC-treated cartilage explants were subjected to mechanical injury, cell death was increased prominently in the superficial zone as compared to explants that were not treated with CABC. To determine mechanisms of increased cell death in CABC treated explants, strain measurements were performed. These results show that the same load (ie 8MPa) cause increased strain (40%) as compared to explants not treated with CABC.

Conclusions: These results indicate that GAG loss alone does not directly lead to chondrocyte death. In response to mechanical injury there is an immediate induction of necrotic cell death that is seen only in GAG-depleted explants and prominent in the superficial zone. This appears to be the result of increased strain in response to mechanical load in the GAG-depleted explants. During subsequent culture cell death spreads via apoptotic mechanisms in CABC and non-CABC treated explants.

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RELATIONSHIP BETWEEN CHONDROCYTE APOPTOSIS AND EXPRESSION OF CARTILAGE EXTRACELLULAR MATRIX MOLECULES

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Purpose: Chondrocyte apoptosis has been suggested to play an important role in the pathogenesis of osteoarthritis (OA). Our previous study has demonstrated a positive association between apoptosis and the microscopic degree of Articular cartilage (AC) damage in equine tissue. Cartilage oligomeric matrix protein (COMP) and fibronectin are important cartilage extracellular matrix (ECM) molecules whcih are known to bind to both the chondrocytes and various components of the ECM. Therefore, alterations in expression of these molecules may have implications for the viability of chondrocytes due to their dependence on attachment to the ECM for maintenance of survival and transfer of critical survival signals between the cell and the ECM. The aim of this study was to determine whether apoptosis is directly associated with expression of COMP and fibronectin in the cartilage ECM.

Methods: AC from the left carpal joint of 12 horses were used in the study. Osteochondral segments were removed from 4 facets (when available) of the articular surface of each joint giving a total of 28 specimens. Apoptotic chondrocytes were identified using an indirect immunohistochemical staining technique to detect the expression of active caspase-3 using a commercially available polyclonal antibody. Immunostaining for COMP and fibronectin was performed using a biotin-streptavidin/peroxidase method using primary antibodies verified to be specific for equine antigens. The intensity of staining for COMP and fibronectin were graded (none, mild, moderate, severe) in each cartilage zone.

Results: We observed a higher rate of apoptosis in cartilage with increased expression of COMP, but this was not statistically significant and there were no differences in the expression of COMP in the different cartilage zones. However, the intensity of fibronectin staining varied according to cartilage zone (superficial$<$middle$<$deep) with significantly increased expression in the deep zone than in either the superficial or middle zones ($P<0.001$). A significant positive association was found between overall intensity of fibronectin staining and overall chondrocyte apoptosis (r=0.44, P=0.0187). The data were also significant for superficial and deep zones (r=0.44, P=0.0239 and r=0.42, P=0.0279 respectively). The correlation between overall intensity of COMP and fibronectin was also significant (r=0.56, P=0.0018).

Conclusions: The positive correlation between the incidence of apoptosis and expression of fibronectin, a key ECM molecule involved in communication between the chondrocyte and surrounding matrix, suggests that chondrocyte death by apoptosis may alter cartilage metabolism and supports the role of this process in the pathogenesis of OA.

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CURCUMIN INHIBITS INTERLEUKIN-6, -8, NITRIC OXIDE AND PROSTAGLANDIN E_2 SYNTHESIS BY BOVINE CHONDROCYTES

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Purpose: This study aimed to investigate the effects of curcumin on the production by primary bovine chondrocytes of nitric oxide (NO), interleukin (IL)-6 and -8 and prostaglandin (PG) E_2.

Methods: Primary bovine chondrocytes were cultured in monolayer until confluence and then incubated for 24h in the absence or in the presence of IL-1beta and with or without curcumin at a concentration ranged between 1 to 30 microM. Cell viability was determined by measuring MTT tetrazolium salt reduction and lactate deshydrogenase release. NO production was assessed by quantifying nitrite in the culture supernatants using the Griess spectrophotometric method. PGE_2 was measured in the culture supernatants by a specific radioimmunoassay. Cyclooxygenase (COX)-1, COX-2, inducible NO synthase (iNOS), IL-6 and IL-8 gene expression were determined by real-time RT-PCR.

Results: Cell viability was not affected by curcumin added alone or in association with IL-1beta. IL-1beta stimulated PGE_2 production and COX-2 gene expression. Curcumin dose dependently inhibited both IL-1beta stimulated COX-2 gene expression and PGE_2 production, but did not affected COX-1 gene expression. IL-6 and IL-8 gene expression were strongly stimulated by IL-1beta. Both IL-1beta stimulated IL-6 and IL-8 gene expression were dose-dependently inhibited by curcumin. Finally, curcumin decreased in a dose-dependent manner IL-1beta stimulated iNOS and NO production by bovine chondrocytes.

Conclusions: Altogether, these in vitro results indicate that curcumin may reduce inflammation and pain in OA by reducing the production of inflammatory mediators by chondrocytes. These findings provide a preclinical basis for the in vivo testing of curcumin and suggest that this natural compound could be helpful to alleviate symptoms in OA patients.